Molecular Cytogenetics of Spiders of Yamuna flood plains of Agra

SYNOPSIS

For the award of the degree of Doctor of Philosophy in Zoology

Submitted by

Km. Hemant Yadav

Supervisor	Head	Dean
Prof. Sant Prakash	Dept. of Zoology	Faculty of Science

MOLECULAR GENETICS LABORATORY
DEPARTMENT OF ZOOLOGY
FACULTY OF SCIENCE
DAYALBAGH EDUCATIONAL INSTITUTE, DAYALBAGH,
AGRA 282005.
MARCH 2016

1. PhD Research Proposal

Molecular Cytogenetics of Spiders of Yamuna flood plains of Agra

The historical city of Agra is based on the banks of the river Yamuna which falls under the semi arid habitat in the South west part of State of Uttar Pradesh and is considered to be the upper part of the Indo Gangetic plains of India. The flood plains of Yamuna, in part of Agra, mainly consist of a vegetation pattern dominated by shrubs and grasses supported by alluvial and residual soil consisting of rocks, pebbles and sand. The climate of Agra faces extreme weather with temperature ranging between 47^0C in summer to as low as 2^0 C in winters. The survival of animals in the flood plains is largely due to their special ability to adapt to these harsh conditions. Spider species apparently are the most successful species in this habitat and are largely unrecorded. Unrevealing the spider diversity and their genetic status is not only a challenge but an urgent need for their conservation.

A comprehensive study on the diversity of spiders suggests that approximately 45000 species of Arachinids, have been recorded globally (Platnick 2013) of which only 810 species are cytogenetically known (Anjali and Prakash 2014; Aroujo *et al*. 2015). In India the spider species reported are largely from the Western Ghats and the rainforest of North eastern part of India and approximately 1686 species have been recorded (Keswani *et al.* 2012) and only 80 species are cytogenetically known (Anjali and Prakash 2015). The record of Spiders from arid/ semi arid habitat are scanty and mostly morphologically based. This group also does not get mentioned in the Scheduled of the Wildlife Protection Act 1972. Only recently their importance in the agriculture as biocontrol of pests and biotechnological applications of its silk secretion and venoms have brought the group in high light. Therefore, studies to build the genetic data base of Spiders across the various habitats are required for the species conservation and utilization.

Polymorphism is a phenomenon occurring mainly due to the necessity for organism to adapt to altering environmental conditions. The strategy of adaptation in such a changing environment consists in the formation of a labile genetic system capable of responding adaptively to a wide range of variable environmental situation. Development in the field of molecular cytogenetics has made it possible in recent years to answer questions concerning the genetic status and the amount of differentiation in and among species. The important fact is that the genes can be selected for study without prior knowledge of variations and the results obtained from their moderate number of loci, can, therefore, be extrapolated to whole of genome.

The paradigm shift towards the study of Molecular Cytogenetics is due to the technological advancements leading to the hundreds and thousands of genetic markers of known loci which can be analyzed in a single organism. The impact of these advancements on genetic studies of adaptations in invertebrates is profound. Cytogenetical information based on chromosome is also essential to the modern taxonomy (Gorlov.*et al*.1995;Macilentus.1999;Fernandez.*et al*.2002; Shorthouse.2003;Danglasde.*et al*.2005;Kral 2007;Stavale.2011 and Kumbicak.2014). A remarkable characteristic of spider chromosomes is the presence of multiple sex chromosomes. The evolution of multiple sex chromosomes in spiders is complicated, and many hypotheses have been proposed (White 1973). However, the X1X2 type seems to

be the most primitive in present day spiders as stated by Suzuki (1954). Karyological studies have been made on spiders of North eastern and eastern part of India (Datta and Chatterjee, 1983) but have lacked behind from other areas of India probably due to the inconsistency in number and size of the chromosomes. The present study will overcome these problems by incorporating molecular based chromosomal techniques such as banding of repetitive DNAs, 28S r RNA and 5S r RNA, and multiple X chromosome patterns for the data base and establishing linkage with the morphological correlates. The proposed study is based on the above objectives and hypothesis and is divided in three sections –

Section 1 Diversity of Araneae in Yamuna Flood plains

Section 2 Cytogenetical studies on the above selected species

Section 3 Biochemical and Molecular studies

2. List of Species reported species of Araneae from Agra region-

Family	species
1.Aranedae	*Argiope sp.*
	Citrophora citricola
2.Lycosideae	*Pradosa sp.*
	Pradosa sp.
	Lycosa sp.
3.Hersilidae	*Hersilia sp.*
4.Oxyopidae	*Oxyopes sp.*
5.Pholsidae	*Artema atlanta*
	Phlocus phalangiodes
6.Saltisidae	*Plexippus paykalli*
	Phidippus sp.
	Hasarius adansoni
7.Thomsidae	*Xysticus sp.*

3. General objective of the study-

To establish the, hitherto unknown, genetic diversity of Spider species in the Yamuna flood plains of Agra and to unravel through molecular cytogenetical tools its genetic labile ability to adapt to the semi arid habitat.

4. Specific Objectives of the study-

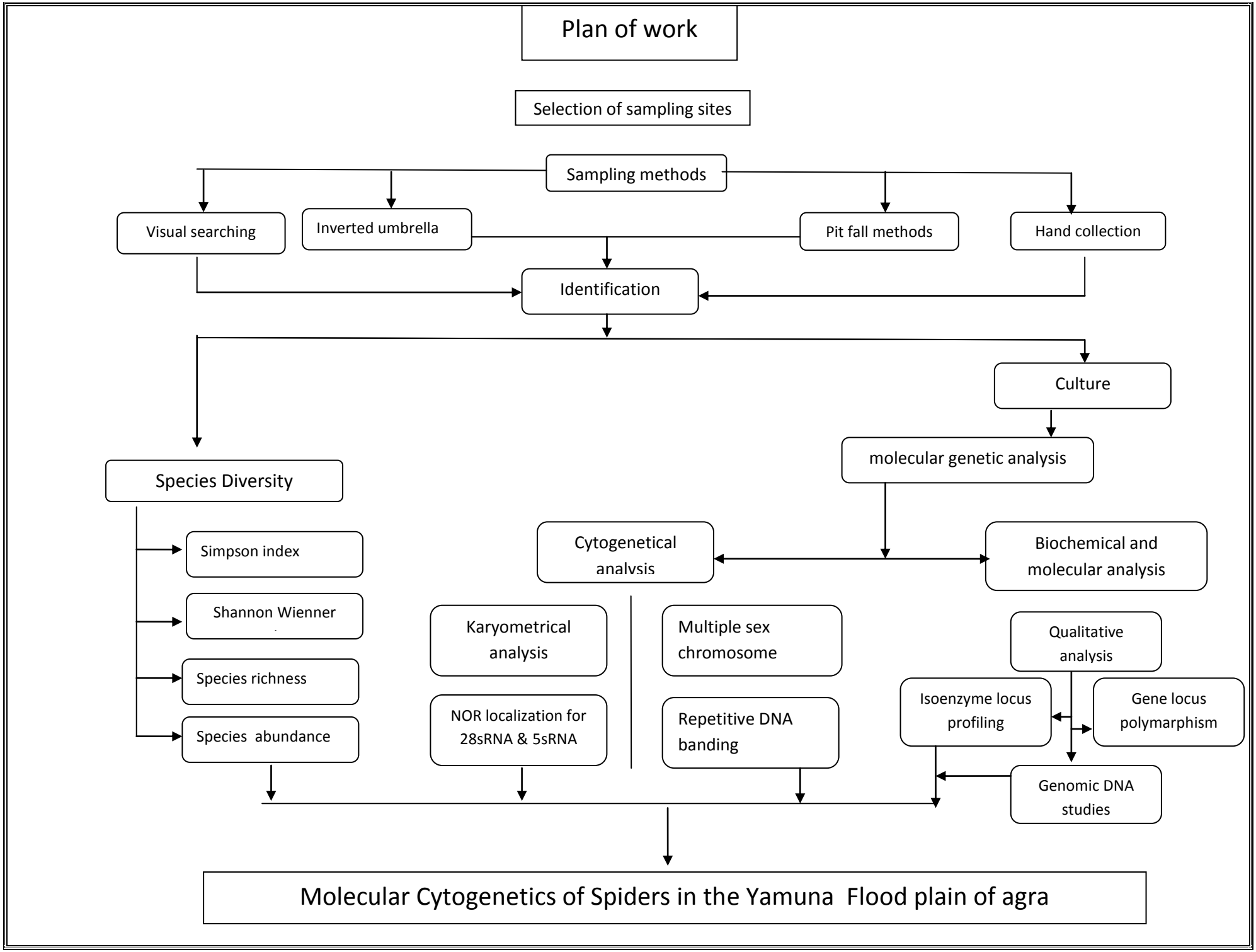
1. To identify and build a database of spider species of Yamuna flood plains.
2. To establish the Karyological status and to establish cytogenetical markers for constitutive repetitive DNA pattern in Aranea
3. To localize 28S and 5 S r DNA in the Nuclear Organsing Regions (NORs).
4. To study the multiple X chromosome system of sex determination in this group.
5. To identify Biochemical genetical markers for the gene loci analyses.
6. Genome studies and utilizing bioinformatic tools for Phylogenetic analyses

5. Protocols-

- Adult Spider Species will be sampled from various sampling sites in the flood plains of Agra by using various innovative methods of collections. Live specimens will be brought to the laboratory for culture and molecular analyses.
- **Spider diversity** will be analysed through various measuring techniques such as Shannon Weiner index; Simpson index and abundance studies.
- **Cytogenetical analysis** will be done by harvesting chromosomes directly from Gonads. Banding technique and NORs will be obtained by suitably modifying various techniques (Smith 1965; Howell and Black 1980; Prakash 1990; Anjali and Prakash 2015).
- **Biochemical and genetical analyses** will be done through Isozyme gene loci linkage using PAGE and various cytochemical staining (Prakash and Chatterjee 1990).
- **Genome analysis** will be done from Hemolymph fluid following Sambrook *et al*. (2001). Genetic softwares and Bioinformatic tools will be utilized for sequence analysis.

6. Progress already made-

Since this is a continuation of an ongoing project to study and build genetic data base of spiders of Northern India. Progress have already been made from the agro- ecosystem habitat in this region, however, the Yamuna flood plains is largely unknown.


Plan of work
Selection of sampling sites
Sampling methods
Visual searching
Inverted umbrella
Pit fall methods
Hand collection
Identification
Culture
molecular genetic analysis
Species Diversity
Simpson index
Shannon Wienner
Species richness
Species abundance
Cytogenetical analysis
Biochemical and molecular analysis
Karyometrical analysis
Multiple sex chromosome
NOR localization for 28sRNA & 5sRNA
Repetitive DNA banding
Qualitative analysis
Isoenzyme locus profiling
Gene locus polymarphism
Genomic DNA studies
Molecular Cytogenetics of Spiders in the Yamuna Flood plain of agra

References-

Araujo,D.,Schneider, M.C.,Paula-Neto, E., Cella,D.M.(2015).The spiders cytogenetic database. Available in www.arthropodacytogenetics.bio.br/spiderdatabase.

Anjali and Prakash(2014) Cytogenetical investigation on spiders of semi-arid areas.Indian Society of Arachnology,ISSN2278-1587.

Bicak,Z.,Ekiz,E. and Cicekil,S.(2014) Karyotype of six spider species belonging to the families Gnaphosidae,salticidae,Thomisidae and Zodariidae (araneae) from Turkey. Comp CytogeneKumt 2014;8(2)93-101.

Sambrock.,J and Maccalume(2000).molecular coloning: a laboratory manual III Edition. Prentice Hall.

Dutta.S.N. and Chaterjee,K.1988: Chromosome and sex determination in 13 araneid spiders of north estern india.Genetica,V(76):91-99

Feernandez. R.,Barragan. M.,Bullejos M,Marchal.J.A.,Guardia,Sanchez(2002).New C-band protocol by heat denaturation in the presence of formide-Heriditas 137:145-148

Howell,W.M., and Black,D.A., (1980),Controlled silver staining of Nucleolar Organiser Regions with a protective colloidal developers: One step method: experentia 36: 1014-1015.

Kambhapati. S.(1995)A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA gene. Proc Natl.Acad. Sci.USA Vol 92,pp2017-1026

Keswani,S.,Hadole,P.and Rajoria,A. (2012) Cecklist of spiders(Aracnida:Agelenidae) from India. Indian jouranal of Arachnology 1(1):-129.

Kumbicak.Z.,(2014) Cytogenetic characterization of ten araneomarph spider (Araneae): karyotype and meiotic fetures. Biologia 69/5:644-650

.

Platnick,N.I.(2013)World Spider Catalog,Version 13.5,.American Museum of Natural History, online at http://research.amnh.org/iz/spiders

Stavale,L.M.,Schneider,M.C.,Brescovit,A.D. and Cella,D.M.,(2011) chromosomal characterstics and karyotype evolution of Oxyopidae spiders (aranae, Entelegynae), Gent.Mol.Res.10(2):752-763.

Suzuki,S.1954:Ctological studies in spiders. III.Studies on chromosomes of fifty seven species of spiders belonging to seventeen families,with general consideration on chromosomal evolution. Journal of Science of the Hiroshima University.Series B.Division 1,v 15(2).23-136.